Available online at www.sciencedirect.com
ScienceDirect
Biological Control 46 (2008) 226–233
Biological Control
www.elsevier.com/locate/ybcon

Application of hydrophilic–lipophilic balance (HLB) number to optimize a compatible non-ionic surfactant for dried aerial conidia of *Beauveria bassiana* ☆

Xixuan Jin [a,*], Douglas A. Streett [a], Christopher A. Dunlap [b], Margaret E. Lyn [a,c]

[a] *USDA-ARS, Biological Control of Pests Research Unit, National Biological Control Laboratory, Mid South Area, 59 Lee Road, P.O. Box 67, Stoneville, MS 38776, USA*

[b] *USDA-ARS, Crop Bioprotection Research Unit, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604, USA*

[c] *USDA-ARS, Application and Production Technology Research Unit, Mid South Area, 141 Experiment Station Road, Stoneville, MS 38776, USA*

Received 3 December 2007; accepted 7 March 2008
Available online 15 March 2008

Abstract

The hydrophilic–lipophilic balance (HLB) number system was used to optimize a compatible non-ionic surfactant, TDA (polyoxyethylene tridecyl ether) in formulations for two *Beauveria bassiana* strains, NI8 and GHA. The optimal HLB number for TDA was determined on the basis of wetting times for conidial powders. The results indicated that optimal HLB number of TDA for *B. bassiana* strain NI8 was 8, while the optimum HLB number for strain GHA was 10. The optimized TDA surfactants required significantly less wetting times than the commonly used laboratory surfactants, Triton X-100, Span 80, and Tween 80. These optimized TDA surfactants were further characterized on their ability to produce conidial suspensions of the two strains after 5 min of mixing, TDA HLB 8 and TDA HLB 10 produced suspensions of 1.8×10^8 and 1.6×10^8 conidia/ml for NI8 and GHA, respectively. These conidial levels were significantly higher than those in Triton X-100, Span 80, and Tween 80 suspensions after the same mixing time. Germination assays showed that TDA HLB 8 promoted significantly higher germination rates of strain NI8 than those observed in other commonly used laboratory surfactants. However, the germination rates of the GHA strain were unaffected by any of the surfactants tested. The efficacy of the conidial suspensions was confirmed with assays against *Lygus lineolaris*. Bioassay results indicated that there were no significant differences in mortalities because of surfactants. These results suggest optimization based upon HLB number will not negatively impact parameters associated with efficacy, while providing desirable physical properties.
Published by Elsevier Inc.

Keywords: *Beauveria bassiana*; Hydrophilic–lipophilic balance; Surfactants; Polyoxyethylene tridecyl ether; Biological control; *Lygus lineolaris*; Tween 80; Triton X-100; Span 80

1. Introduction

Beauveria bassiana (Balsamo) Vuillemin is one of the most promising entomopathogenic fungi for commercialization. According to Faria and Wraight (2007), *B. bassiana* products comprise 33.9% of the total mycoinsecticides developed worldwide targeting almost all economically important insects distributed in Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Isoptera, etc. Laboratory and field studies have shown *B. bassiana* to be effective against a variety of crop and storage pests (Leathers and Gupta, 1993; Wraight et al., 1998; De La Rosa et al., 2000; Lacey et al., 2001; Akbar et al., 2005; Lord, 2005; Al-mazra'awi et al., 2006; Leland and McGuire, 2006; Athanassiou and Steenberg, 2007; Hansen and Steenberg, 2007), grasshoppers (Khachatourians, 1992), termites (Culliney and Grace,

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

* Corresponding author. Fax: +1 662 686 5281.
E-mail address: xixuan.jin@ars.usda.gov (X. Jin).

1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2008.03.008

2000), house flies (Kaufman et al., 2005), mosquitoes (Clark et al., 1968), ticks (Kirkland et al., 2004), fire ants (Broome et al., 1976; Pereira et al., 1993), and mites (Wekesa et al., 2005). Mass production techniques have been well documented for *B. bassianna* (Grimm, 2001; Bradley et al., 1992, 2002). Large quantities of aerial conidia have been produced with either solid or biphasic fermentation, the latter becoming more common in recent years.

Aerial conidia, especially dried conidia of *B. bassianna* are hydrophobic, and surfactants are needed for water-based formulations in laboratory studies, greenhouse bioassays, and field trials as well as commercial formulation developments. A variety of surfactants has been used to either harvest aerial conidia of *B. bassiana* from the culture plates or to prepare conidial suspensions for bioassays and field trials, including TDA (polyoxyethylene tridecyl ether) (Jin et al., 1999), Tween 80 and 20 (polyoxyethylene sorbitan monostearate and polyoxyethylene sorbitan monolaurate) (Wraight et al., 1998; Kirkland et al., 2004; Leland et al., 2005; Al-mazra'awi et al., 2006; Lazzarini et al., 2006), Span 80 (sorbitan monooleate) (Luz and Batagin, 2005), Triton X-100 (polyoxyethylene isooctylphenyl ether) (Leathers and Gupta, 1993; Kouassi et al., 2003; Wekesa et al., 2005), and Silwet L-77 (Brownbridge et al., 2001; James and Elzen, 2001; Akbar et al., 2005; Ugine et al., 2005). TDA is a non-ionic surfactant, and has been used in commercial formulations of biological control products (Jin et al., 1999). Tween 80, Tween 20, Span 80 and Triton X-100 are also non-ionic, and are generally used as laboratory wetting agents.

Compatibility between surfactants and *B. bassiana* has been previously studied (Johal and Marold, 1996; Morales and Rochling, 1998; Jin et al., 1999; Luz and Batagin, 2005), and surfactants have been used to obtain long term stability of conidia of *B. bassiana* (Johal and Marold, 1996). In addition, non-ionic surfactants have been generally considered to be less toxic to microorganisms than ionic surfactants (Luz and Batagin, 2005), which make them ideal candidates for use in water-based conidial suspensions.

Non-ionic surfactants consist of a molecule that combines both hydrophilic and lipophilic groups (or polar and non-polar groups) and it is the balance of the size and strength of these two opposing groups that is called the hydrophilic–lipophilic balance (HLB) number (Griffin, 1949, 1954). Calculation of the HLB value for non-ionic surfactants was established by Griffin (1954). In Griffin's system, a surfactant that is lipophilic in character is assigned a low HLB number and a surfactant that is hydrophilic in character is assigned a high number. Griffin's method is satisfactory for non-ionic surfactants of various chemical groups. However, two surfactants may have the same HLB number but exhibit different wetting characteristics because of the differences existing in chemical groups. Subsequently, new methods to determine HLB number have been developed (Davies, 1957; Ben-Et and Tatarsky, 1972; Rabaron et al., 1993), and now we can determine the HLB numbers for surfactants that are not in non-ionic groups.

The HLB number for optimizing surfactants has been applied to almost all industries wherever surfactants are needed in product development, including pharmaceutical, food, cosmetic, pesticide, and herbicide formulations. However, less research has been conducted in microbial pesticide development. Jin et al. (1999) reported using TDA with certain HLB numbers to reactivate dried conidia of *Metarhizium anisopliae* after long term storage in the development of commercial formulations. Procedures applying the HLB number in optimizing a compatible surfactant for hydrophobic conidia are different from those for chemicals. The focus must be placed on both physical and biological properties. Empirical data from more than a decade of research in formulating hydrophobic microbial conidia in our laboratories suggest the HLB number is a convenient tool in surfactant selection and optimization. This report describes the procedures used to apply the HLB number in optimizing a compatible non-ionic surfactant, TDA for two *B. bassiana* strains. Optimization of the surfactant included investigations on (1) physical properties, i.e., required wetting time and conidial counts in a suspension after a fixed time of mixing, and (2) biological properties, conidial germination and efficacy in comparison with other laboratory used surfactants on tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois).

2. Materials and methods

2.1. Beauveria bassiana strains

Two *B. bassiana* strains NI8 and GHA were used in this study. Conidia of strain NI8 and strain GHA were provided by the USDA-ARS, Southern Insect Management Research Unit, Stoneville, MS. *B. bassiana* strain NI8 was originally isolated from *L. lineolaris* on Horseweed in Washington County, Mississippi (Leland et al., 2005), and is highly pathogenic to plant bugs (Leland et al., 2005; Leland and McGuire, 2006). *B. bassiana* strain GHA is a commercial strain owned by Laverlam International Corporation, Butte, MT (formerly Emerald BioAgriculture Corporation). Conidia of strain GHA was originally provided by Emerald BioAgriculture Corporation to the Southern Insect Management Research Unit as technical powder. Conidia of these two strains were placed in plastic bottles and stored in the refrigerator at 4 °C for more than 12 months before this study. Because conidial biomass contained some fine starch particles that interfered with this study, conidia were passed through a 400 mesh sieve before use. Conidia of NI8 were dried in paper bags at 26 °C until the moisture content reached 6–7%, and GHA was provided as technical powder and its drying conditions were unknown. Moisture contents of conidia of these two strains were determined using an HB 43 Halogen Moisture Analyzer (Mettler-Toledo

GmbH, Laboratory & Weighing Technologies, CH-8606 Greifensee, Switzerland). The moisture content levels were 6.5% for NI8 and 5.1% for GHA, respectively. Conidial germination was evaluated on potato dextrose agar (PDA, Becton, Dickinson and Company, Sparks, MD) plates. Dry conidia were directly spread onto PDA by sterile cotton swabs (Akbar et al., 2005), and incubated at 26 °C for 20 h. Germination percentages were 59% and 92% for N18 and GHA, respectively.

2.2. Wetting time

Surfactants used in this study were Ethal TDA (polyoxyethylene tridecyl ether, Ethox Chemical, LLC, Greenville, S.C.), Tween 80 (polyoxyethylene sorbitan monostearate, Fisher Scientific, Fair Law, NJ), Span 80 (sorbitan monooleate, Sigma–Aldrich Inc., St. Louis, MO) and Triton X-100 (polyoxyethylene isooctylphenyl ether, LabChem Inc. Pittsburgh, PA). TDA surfactants are commercially available with different HLB numbers, and through combining them as mixtures we were able to produce an intermediate HLB number surfactant. TDA with HLB number below 8 was not commercially available, and therefore TDA 3 (HLB = 8) and TDA 9 (HLB = 13) were used to make TDA mixtures of HLB 9, 10, 11, and 12 according to Griffin's formula (1949).

The wetting study was performed using TDA mixtures with HLB numbers of 8, 9, 10, 11, and12 in comparison with other generally used laboratory surfactants, Tween 80 (HLB = 15), Triton X-100 (HLB = 13.6) and Span 80 (HLB = 4.3) (Gennis and Strominger, 1976; Kruglyakov et al., 2002). Each surfactant was one treatment and had three replicates with each replicate consisting of three samples (flasks). Three 0.1-g samples of conidia were added to three 250 ml Erlenmeyer flasks, each containing 100 ml of 0.2% surfactant solution in deionized (DI) water. In our preliminary works, several surfactant concentrations were tested, and 0.2% solution was the most suitable one to conduct this study. Flasks were placed on a rotary shaker (Eberbach Scientific Instruments & Apparatus USA) and then shaken at 125 rpm (Al-mazra'awi et al., 2006) under a fume hood at room temperature until all macroscopically visible clusters of conidia were fully suspended and the wetting time recorded.

2.3. Conidial counts

Surfactants used for strain NI8 were TDA HLB 8, Tween 80, Span 80 and Triton X-100, and those for strain GHA were TDA HLB 10, Tween 80, Span 80, and Triton X-100. A 0.1-g sample of conidia was transferred to a 250 ml Erlenmeyer flask, containing 100 ml of 0.2% of each surfactant in DI water. The flask was placed on the rotary shaker and shaken at 125 rpm under a fume hood at room temperature for 5 min. A 1-ml sample was collected from the center of the flask and then diluted to the countable level in sterile DI water. A Neubauer Brightline Hemacytometer (Hausser Scientific Co., Horsham, Pa) was used to count conidia present, and the total conidia number per milliliter was calculated.

2.4. Germination studies

Surfactants used in the germination study of strains NI8 and GHA were the same ones used in the conidial counts study. A sterile cotton swab was used to touch the conidia, and then placed in a test tube containing 3 ml of 0.2% surfactant solution. The test tube and swab were vortexed for 10 s. The swab was then used to spread the conidia onto one quadrant of a PDA plate. A new sterile swab was used to spread the conidia from one part to the next until the third part where the germination rate could be determined.

The plates were incubated at 26 °C. Germination was counted at 12, 14, 16, and 18 h for strain NI8, and at 8, 10, 12, 14, and 16 h for strain GHA. Successful germination was recorded when the germ tube was present and equal to or longer than the conidial length. Three plates were counted by observing 200 conidia from different areas on each plate.

2.5. Colony

The *L. lineolaris* colony used in this study was from a colony established from field collections in Mississippi by the Biological Control of Pests Research Unit, USDA-ARS. The colony has been maintained on the NI diet (Cohen, 2000) for over 5 years. Rearing conditions were 25 °C, 50% RH, and a photoperiod of 16:8 (L:D) in the National Biological Control Laboratory, USDA-ARS, Stoneville, MS.

2.6. Bioassay

The *B. bassiana* strain NI8 was used in the bioassay and followed the procedures described by Leland et al. (2005) with modifications to investigate the efficacy of conidia suspended in different surfactants on adult tarnished plant bugs. Bioassays were conducted at 4 concentration levels of viable conidia, 0, 10^5, 10^6, and 10^7/ml in 0.05% surfactant solutions of DI water. Surfactants used were TDA HLB 8, Triton X-100, Span 80 and Tween 80. Adult *Lygus* were temporarily incapacitated by CO_2, and 30 bugs were placed in a 9 cm diameter Petri dish lined with filter paper which was wetted with sterile DI water. The dish was then transferred to a spray tower. Test tubes containing different concentrations of viable conidia were votexed for 10 s before spray, and 1 ml of conidia suspension was sprayed onto the bugs. Individual bugs were then transferred to a transparent 30 ml medicine cup (Southern Container Corp., Mooresville, N.C.) with a piece of Aquafoam (Syndicate Sales, Inc., Kokomo, IN) on the bottom that was saturated with 10% SueBee Clover honey (Packed by Sioux Honey Ass'n General Office: Sioux City, Iowa) solution. Each cup was capped with a hard paper lid (L.P.C. Corp.,

Hairfield, NJ). The bugs in individual cups were kept in an incubation room at 28 °C and 70% relative humidity for 10 days. Bugs which died within 24 h after treatment were excluded from analysis. Only cadavers with conidiating *B. bassiana* recorded at the 5th, 7th and 10th days were included in the mortality analysis.

2.7. Statistical analysis

All treatments in a replicate were evaluated at the same time and were repeated three times. Data were log-transformed in wetting time and conidial counts studies. All data were subjected to ANOVA, Mixed Models analysis, and the Least Square Post Hoc tests to identify significant differences among treatments at 0.05 levels (Littell et al., 2006). The bioassay was a randomized complete block with factorial treatment structure of 4 concentrations of conidia × 4 surfactants. For bioassay data, equality of variance assumption was also tested between surfactant and concentration groups using Null Model Likelihood Ratio Test in SAS Proc Mixed Software (Littell et al., 2006).

3. Results

3.1. Wetting time

The results showed that TDA surfactants provided faster wetting times for both strains relative to the other surfactants tested (Fig. 1). The Tween 80 solution was the only surfactant which failed to completely wet the samples of both strains under these conditions and the experiment was discontinued after 8 h. The Tween 80 solution also produced foaming during mixing, which was a disadvantage when making conidial suspensions. Conidia of *B. bassiana* strain GHA were found to be suspended faster than strain NI8 for each of the surfactants tested (Fig. 1).

The effect of HLB on the TDA series for each strain did not show a trend (Fig. 1). ANOVA indicated significant differences between surfactant treatment means (strain NI8: $F = 1448.76$, $P < 0.0001$, $df = 62$; strain GHA: $F = 1576.06$, $P < 0.0001$, $df = 62$). The fastest wetting time observed for *B. bassiana* strain NI8 occurred with the TDA HLB 8. The average time needed to wet a conidial preparation of strain NI8 by TDA HLB 8 was 3.2 min, which was significantly less ($P < 0.01$) than the wetting times needed by the other TDA HLB numbers and the other surfactants. The fastest wetting occurred with HLB 10 for *B. bassiana* strain GHA. The average time required for HLB 10 to wet the conidia of strain GHA was 1.2 min, which was significantly less ($P < 0.01$) than wetting times needed by using TDA HLB 8, 9, 11 and 12, and the other surfactants.

3.2. Conidial counts

The results of conidial counts were presented in Table 1. There were significant differences between surfactant treat-

Table 1
Conidial counts of *Beauveria bassiana* in 100 ml of 0.2% common laboratory used surfactants, and 0.2% polyoxyethylene tridecyl ether (TDA) solutions with an optimal hydrophilic–lipophilic Balance (HLB) number after 5 min shaken on a rotary shaker at 125 rpm

B. Bassiana strain	Surfactant	Average log conidia counts	Std. error	Statistical significance ($P < 0.0001$)
NI8	TDA HLB 8	8.4	0.01	a
	Triton X-100	8.2	0.02	b
	Span 80	7.4	0.03	c
	Tween 80	7.2	0.08	d
GHA	TDA HLB 10	8.2	0.02	a
	Triton X-100	8.0	0.02	b
	Span 80	7.4	0.05	c
	Tween 80	6.9	0.02	d

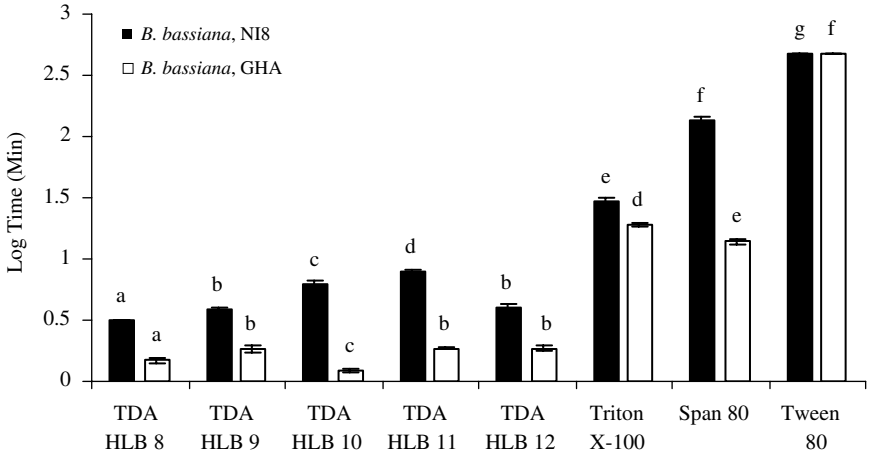

Fig. 1. Times required for wetting 0.10 g dry conidia of *Beauveria bassiana* in 100 ml of 0.2% polyoxyethylene tridecyl ether (TDA) solutions with different hydrophilic–lipophilic balance (HLB) numbers and 0.2% solutions of other common laboratory used surfactants shaken at 125 rpm at room temperature. [a]Values are the means of three experiments; bars represent standard errors. For a given isolate means with different letters within each *B. bassiana* strain are significantly different ($P < 0.01$).

ment means of both strains (strain NI8: $F = 418.68$, $P < 0.0001$, df = 30; strain GHA: $F = 430.01$, $P < 0.0001$, df = 30) on conidial counts. In general, the conidial concentration levels of both strains in the suspensions were in the order of TDA HLB 8 or TDA HLB 10 > Triton X-100 > Span 80 > Tween 80. For strain NI8, TDA HLB 8 suspension had significantly ($P < 0.0001$) higher conidia concentration (1.8×10^8 conidia/ml) than Triton X-100, Span 80 and Tween 80, while TDA HLB 10 solution provided the highest ($P < 0.0001$) concentration of conidia (1.6×10^8 conidia/ml) for the GHA strain.

3.3. Germination study

The impact of the surfactants on conidia viability was determined by germination studies. For strain N18, conidial germination wetted by TDA HLB 8 was significantly higher than the germination percents of conidia suspended by other general used laboratory surfactants, Tween 80, Span 80 and Triton X-100 throughout the time course of collection ($F = 21.69$, $P < 0.0001$, df = 30 at 12 h; $F = 5.20$, $P < 0.0115$, df = 30 at 14 h; $F = 3.97$, $P < 0.0169$ at 16 h; $F = 93.20$, $P < 0.0001$, df = 30 at 18 h, respectively) (Fig. 2). The germination percent became uncountable after 18 h of incubation due to over growth of germ tubes in all treatments. For strain GHA, conidia germination remained the same across all treatments (Fig. 2). There were no significant ($P > 0.05$) differences in germination among all treatments or time points. The germination became uncountable after 16 h.

3.4. Bioassay

There were neither significant differences ($P > 0.05$) in mortalities across all treatments nor significant differences ($P > 0.05$) between conidial concentration levels in the first 5 days (data not shown). After 7 days of treatment, no significant differences ($P > 0.05$) were observed in mortality among the surfactants used in this study (Fig. 3). Mortality showed significant dose response ($F = 84.84$, $P < 0.0001$, df = 39) regardless of surfactants. The treatments with 10^7, 10^6, and 10^5 conidia/ml in TDA HLB 8 resulted in *Beauveria* conidiation on 71.5%, 36.7%, and 2.5% of dead tarnished plant bugs, respectively. Mortalities caused by 10^7, 10^6, and 10^5 conidia/ml in the suspensions of Triton X-100, Span 80 and Tween 80 followed the same trend of TDA HLB 8. No significant interactions ($P > 0.05$) between surfactant and conidial concentration levels were detected.

Results observed at day 10 were presented in Fig. 3. Data analysis indicated there were no significant differences ($P > 0.05$) in mortalities among surfactants. The trend of mortalities caused by conidial concentration levels at day 10 was similar to the results obtained at day 7. Dose response was significant ($F = 212.26$, $P < 0.0001$, df = 39). Mortalities caused by 10^7 conidia/ml were significantly higher ($P < 0.0001$) than the ones by 10^6 conidia/ml, and 10^6 conidia/ml resulted in significantly higher ($P < 0.0001$) mortalities than 10^5 conidial/ml did, regardless of the surfactants. No significant interactions ($P > 0.05$) between surfactant and conidial concentration levels were detected.

4. Discussion

Results from this study demonstrated that the HLB number could be used to optimize surfactants for conidial suspensions. In general, surfactants with lower HLB numbers (4–6) are mostly used as emulsifiers (water-in-oil), while those with higher HLB numbers (13–15) are detergents, and surfactants with HLB numbers between 7 and 9 are superior candidates for wetting agents (Griffin, 1949). Results reported in this study for optimum surfactants were in the HLB wetting range. HLB numbers for Triton X-100 and Tween 80 are 13.6 and 15.0, respectively. They were in the range of detergents, and prone to foaming. Foam formation should always be avoided because it reduces the uniformity of the conidial suspension. Span

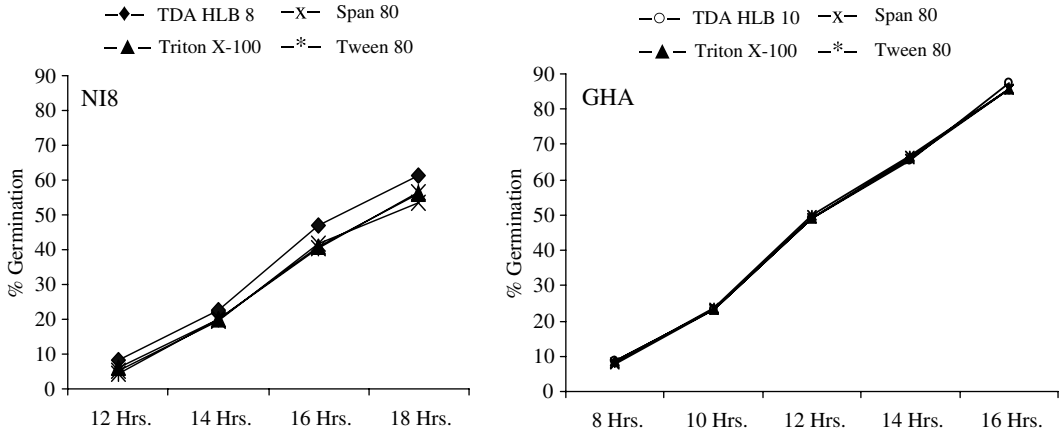

Fig. 2. Conidia germinations of *Beauveria bassiana* wetted by 0.2% solutions of common laboratory used surfactants and polyoxyethylene tridecyl ether (TDA) with an optimal hydrophilic–lipophilic balance number on PDA plates incubated at 26 °C and observed at different incubation times.

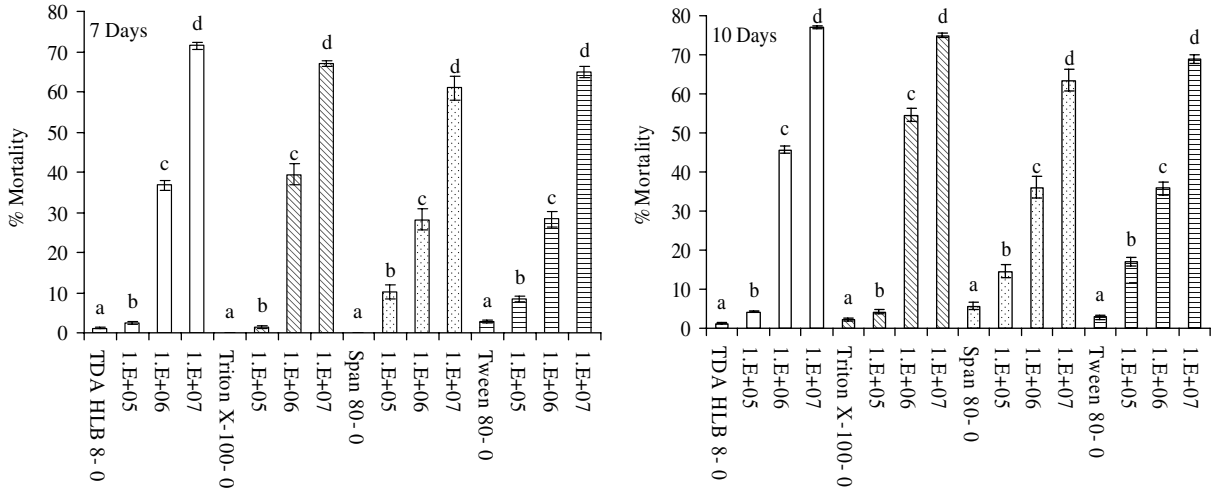

Fig. 3. Mortality of tarnished plant bugs treated with four viable conidial concentrations/ml of *Beauveria bassiana* strain NI8 in 0.05% common laboratory used surfactants and polyoxyethylene tridecyl ether (TDA) with an optimal hydrophilic–lipophilic balance (HLB) number. [a]Values are the means of three experiments; bars represent standard errors. Means with different letters within each surfactant are significantly different ($P < 0.0001$).

80 has a HLB number of 4.3, and was in the range of water-in-oil emulsifiers, which were intended to stabilize water droplets in oil. Understanding these basic HLB guidelines simplifies the surfactant selection for biological control applications. In the case of conidial suspensions, we were primarily interested in using the surfactant as a wetting agent. Following the HLB guidelines, we can quickly eliminate Triton X-100, Tween 80, and Span 80 from consideration, since they were outside the HLB range for wetting agents.

Once a potential surfactant has been chosen using the HLB guidelines, its performance can be tested and optimized by assessing some simple physical properties. The results showed how wetting time, an easily measurable parameter, could be used to optimize the physical properties of the system. TDA is a convenient surfactant for this application because its HLB numbers can be tailored to a wide range of values and tested. In this report we showed strain NI8 had an optimum HLB number of 8, while GHA had an optimum HLB number of 10, based on wetting times. Wetting times reflect the chemical interactions between the surfactant and the conidia. Faster wetting times infer favorable chemical interactions, which are important in forming uniform suspensions. Our results showed that the surfactant with the fastest wetting times also had the highest conidia concentrations after a fixed mixing time. The quantity of suspended conidia in a surfactant solution is a critical issue of commercial product development because higher conidial concentration levels should improve efficacy. All of these results suggested that different strains of *B. bassiana* might use the same non-ionic surfactant, but with different HLB numbers to optimize physical properties required for formulation development and application improvement. This finding provides us with a method to select a compatible non-ionic surfactant, and determine the optimal HLB number for a specific strain which is produced under certain defined conditions. The differences in the optimal HLB numbers among strains of *B. bassiana* may be related to strain physicochemical characteristics, such as cell wall hydrophobicity. Moreover, different production systems, growth media, conidial ages and even moisture levels may also result in changes of the surface characteristics of the conidia (Hegedus et al., 1990, 1992). It was obvious that strains NI8 and GHA were produced and processed in different systems, and had moisture contents at 6.5% and 5.1%, respectively. Our unpublished data showed that if moisture differences were greater than 10%, then moisture content would affect wetting time. The moisture content difference between NI8 and GHA in this study was not appreciable to detect differences in wetting time in these studies.

There are some other surfactants used in biological control programs, such as Silwet L-77 (Brownbridge et al., 2001; James and Elzen, 2001; Akbar et al., 2005; Leland et al., 2005; Ugine et al., 2005). Silwet L-77 is an organosilicon surfactant. Its HLB number can not be calculated by using Griffin's method. However, it can be measured by using the Cloud Point method. We would predict that the HLB number of Silwet L-77 is around 8–10, or more probably 9–10. This is based on its performance in biological control applications where it is an effective wetting agent.

In the commercial development of a biological pesticide, conidia are usually dried to a low moisture content level to induce dormancy prior to formulation and long term storage. Surfactants are used to end this dormancy and to promote germination in water-based applications. Germination is an indicator of conidial viability and vigor. Conidial vigor, as distinguished from viability, relates to the relative "strength" or "weakness" of conidial germination and germ tube growth (Jin et al., 1992), and is one of the important characteristics of a biological control strain. Conidial vigor can be weakened by processing and long term storage, and causes slow germination which is poorly synchronized (Jin et al., 1992). Our results showed that

dried conidia of *B. bassiana* strain GHA had much greater vigor than strain NI8. The initial germination percents of strains NI8 and GHA were 59% and 92%, respectively. Conidia of NI8 might lose more reserved materials, such as sugars, or had more damage to their cell components during processing and long term storage than strain GHA, and the vigor of strain NI8 conidia was weakened. But the conidia were not dead. The optimized surfactant, TDA HLB 8 promoted quick wetting, better water absorption and activation. Therefore, germination of NI8 suspended by TDA HLB 8 was significantly higher as compared to other surfactants in this study. Jin et al. (1999) also reported the improved germination rate of *M. anisopliae* conidia after long term storage using TDA HLB 10. GHA is a commercially available strain that has been through a comprehensive screening process, and possesses the ability to maintain conidia vigor after processing and long term storage. Consequently, the germination of strain GHA was more rapid, higher and better synchronized than the germination of strain NI8, regardless of surfactants. Our results indicated that optimizing the surfactant could improve the germination of hydrophobic conidia of *B. bassiana* strains which had low vigor. Conidial vigor is a comprehensive reflection of conidial physiological conditions after production, harvesting, drying, formulation and long term storage, and should be considered as one of the important characteristics that a commercial strain must possess.

Bioassay results observed at day 5, 7, and 10 indicated that there were no significant differences in efficacy between the surfactants tested. Conidia concentration level was the sole factor that governed efficacy. This suggests if a compatible surfactant is selected, further optimization using the HLB system should have no negative impact on efficacy. The rate of germination of strain NI8 was promoted by TDA HLB 8 in the germination study. However, this was not reflected in improved efficacy in the bioassay study. Germination study was conducted on PDA plates under time restriction (18 h) due to over growth of germ tubes. Benomyl-based medium (Meikle et al., 2003) was not used in this study to inhibit germ tube elongation and to postpone incubation time. Therefore, final readings of conidial germination of strain NI8 might not represent the total germinable conidia. Optimized surfactant might only speed up germination of strain NI8, but did not improve the total germination rates. Moreover, the time course for germination (18 h) was short compared with the time scale for the infection and conidiation processes (5+ days). Therefore, the advantage in germination was not observed in bioassay efficacy under these conditions.

According to Griffin's theory (1954), to select a surfactant properly for any application, one must have the optimal HLB value and the correct chemical group. This has been ignored for some time, and today we are still using some "correct chemical group" surfactants without the right HLB or "incorrect chemical group" surfactants with the right HLB in our laboratory practices and bioassays involving hydrophobic conidia. The HLB classification system offers some prediction of behavior and reduces the labor required to select the optimal surfactant for both laboratory studies and product development of hydrophobic biological control agents.

Acknowledgments

The technical assistance of Robert B. Elliott is gratefully acknowledged. We thank Debbie Boykin, USDA-ARS-MSA, Stoneville, MS for providing the statistical assistance. We also thank Gerald J. Gipson, Tabatha R. Ramsey, Lisa L. Self, and Goulei Feng for their assistance in conducting the bioassay.

References

Akbar, W., Lord, J.C., Nechols, J.R., Loughin, T.M., 2005. Efficacy of *Beauveria bassiana* for red flour beetle when applied with plant essential oils or in mineral oil and organosilicone carriers. Journal of Economic Entomology 98, 683–688.

Al-mazra'awi, M.S., Shipp, L., Broadbent, B., Kevan, P., 2006. Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper. Biological Control 37, 89–97.

Athanassiou, C.G., Steenberg, T., 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L) (Coleoptera: Curculionidae). Biological Control 40, 411–416.

Ben-Et, G., Tatarsky, D., 1972. Application of NMR for the determination of HLB values of nonionic surfactants. Journal of the American Oil Chemists' Society 49, 499–500.

Bradley, C.A., Black, W.E., Kearns, R., Wood, P., 1992. Role of production technology in mycoinsecticide development. In: Leatham, G.F. (Ed.), Frontiers in Industrial Mycology. Chapman & Hall, Inc., New York, NY, pp. 160–173.

Bradley, C.A., Wood, P.P., Black, W.E., Kearns, R.D., Britton, J., 2002. Solid culture substrate including barley. US Patent Application No. 20020006650.

Broome, J.R., Sikorowski, P.P., Norment, B.R., 1976. A mechanism of pathogenicity of *Beauveria bassiana* on larvae of the imported fire ant, *Solenopsis richteri*. Journal of Invertebrate Pathology 28, 87–91.

Brownbridge, M., Costa, S., Jaronski, S.T., 2001. Effects of *in vitro* passage of *Beauveria bassiana* on virulence to *Bemisia argentifolii*. Journal of Invertebrate Pathology 77, 280–283.

Clark, T.B., Kellen, W.R., Fukuda, T., Lindegren, J.E., 1968. Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. Journal of Invertebrate Pathology 11, 1–7.

Cohen, A.C., 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois). Journal of Entomological Science 35, 301–310.

Culliney, T.W., Grace, J.K., 2000. Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus*. Bulletin of Entomological Research 90, 9–21.

Davies, J.T., 1957. A quantitative kinetic theory of emulsion type, I. Physical chemistry of the emulsifying agent, gas/liquid and liquid/liquid interface. In: Proceedings of the International Congress of Surface Activity, pp. 426-438.

De La Rosa, W., Alatorre, R., Barrera, J.F., Toriello, C., 2000. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon

the coffee berry borer (Coleoptera: Scolytidae) under field conditions. Journal of Economic Entomology 93, 1409–1414.

Faria, M.R. de, Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43, 237–256.

Gennis, R.B., Strominger, J.L., 1976. Activation of C_{55}-isoprenoid alcohol phosphokinase from *Staphylococcus aureus*. The Journal of Biological Chemistry 251, 1277–1282.

Griffin, W.C., 1949. Classification of surface-active agents by "HLB". Journal of the Society of Cosmetic Chemists 1, 311–326.

Griffin, W.C., 1954. Calculation of HLB values of non-ionic surfactants. Journal of the Society of Cosmetic Chemists 5, 249–256.

Grimm, C., 2001. Economic feasibility of a small-scale production plant for entomopathogenic fungi in Nicaragua. Crop Protection 20, 623–630.

Hansen, L.S., Steenberg, T., 2007. Combining larval parasitoids and an entomopathogenic fungus for biological control of *Sitophilus granarius* (Coleoptera: Curculionidae) in stored grain. Biological Control 40, 237–242.

Hegedus, D.D., Bidochka, M.J., Khachatourians, G.G., 1990. *Beauveria bassiana* submerged conidia production in a defined medium containing chitin, two hexosamines or glucose. Applied Microbiology and Biotechnology 33, 641–647.

Hegedus, D.D., Bidochka, M.J., Miranpuri, G.S., Khachatourians, G.G., 1992. A comparison of the virulence, stability and cell-wall-surface characteristics of three spore types produced by the entomopathogenic fungus *Beauveria bassiana*. Applied Microbiology and Biotechnology 36, 785–789.

James, R.R., Elzen, G.W., 2001. Antagonism between *Beauveria bassiana* and imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. Journal of Economic Entomology 94, 357–361.

Jin, X., Hayes, C.K., Harman, G.E., 1992. Principles in the development of biological control systems employing Trichoderma species against soil-borne plant pathogenic fungi. In: Leatham, G.F. (Ed.), Frontiers in Industrial Mycology. Chapman & Hall, Inc., New York, NY, pp. 174–195.

Jin, X., Grigas, K.E., Johnson, C.A., Perry, P., Miller, D.W., 1999. Method for storing fungal conidia. US Patent 5, 989, 898.

Johal, S.S., Marold, L.M., 1996. Maintenance and long term stabilization of fungal conidia using surfactants. US Patent 5, 512, 280.

Kaufman, P.E., Reasor, C., Rutz, D.A., Ketzis, J.K., Arends, J.J., 2005. Evaluation of *Beauveria bassiana* applications against adult house fly, *Musca domestica*, in commercial caged-layer poultry facilities in New York state. Biological Control 33, 360–367.

Khachatourians, G.G., 1992. Virulence of five *Beauveria* strains, *Paecilomyces farinosus*, and *Verticillium lecanii* against the migratory grasshopper, *Melanoplus sanguinipes*. Journal of Invertebrate Pathology 59, 212–214.

Kirkland, B.H., Westwood, G.S., Keyhani, N.O., 2004. Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to Ixodidae tick species *Dermacentor variabilis, Rhipicephalus sanguineus*, and *Ixodes scapularis*. Journal of Medical Entomology 41, 705–711.

Kouassi, M., Coderre, D., Todorova, S.I., 2003. Effects of the timing of applications on the incompatibility of three fungicides and one isolate of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina). Journal of Applied Entomology 127, 421–426.

Kruglyakov, P.M., Mal'kov, V.D., Sedova, T.P., 2002. The effect of fatty alcohols and valeric acid on the regions of existence of a three-phase system containing water, oil, and surfactant. Colloid Journal 64, 719–724.

Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? Biological Control 21, 230–248.

Lazzarini, G.M.J., Rocha, L.F.N., Luz, C., 2006. Impact of moisture on *in vitro* germination of *Metarhizium anisopliae* and *Beauveria bassiana* and their activity on *Triatoma infestans*. Mycological Research 110, 485–492.

Leathers, T.D., Gupta, S.C., 1993. Susceptibility of the eastern tent caterpillar (*Malacosoma americanum*) to the entomogenous fungus *Beauveria bassiana*. Journal of Invertebrate Pathology 61, 217–219.

Leland, J.E., McGuire, M.R., Grace, J.A., Jaronski, S.T., Ulloa, M., Park, Y.H., Plattner, R.D., 2005. Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera: Miridae). Biological Control 35, 104–114.

Leland, J.E., McGuire, M.R., 2006. Effects of different *Beauveria bassiana* isolates on field populations of *Lygus lineolaris* in pigweed (*Amaranthus* spp.). Biological Control 39, 272–281.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS for Mixed Models, Second ed. SAS Institute Inc., Cary, NC.

Lord, J.C., 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biological Control 34, 180–186.

Luz, C., Batagin, I., 2005. Potential of oil-based formulations of *Beauveria bassiana* to control *Triatoma infestans*. Mycopathologia 160, 51–62.

Meikle, W.G., Jaronski, S.T., Mercadier, G., Quimby, P.C., 2003. A distributed delay routine-based simulation model of *Beauveria bassiana* conidial stability in response to environmental stressors. BioControl 48, 561–578.

Morales, E., Rochling, H., 1998. Water-dispersible granules of spores or live *Beauveria bassiana*. US Patent 5, 730, 973.

Pereira, R.M., Stimac, J.L., Alves, S.B., 1993. Soil antagonism affecting the dose-response of workers of the red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. Journal of Invertebrate Pathology 61, 156–161.

Rabaron, A., Cavé, G., Puisieux, F., Seiller, M., 1993. Physical methods for measurement of the HLB of ether and ester non-ionic surface-active agents: H-NMR and dielectric constant. International Journal of Pharmaceutics 99, 29–36.

Ugine, T.A., Wraight, S.P., Sanderson, J.P., 2005. Acquisition of lethal doses of *Beauveria bassiana* conidia by western flower thrips, *Frankliniella occidentalis*, exposed to foliar spray residues of formulated and unformulated conidia. Journal of Invertebrate Pathology 90, 10–23.

Wekesa, V.W., Maniania, N.K., Knapp, M., Boga, H.I., 2005. Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*. Experimental and Applied Acarology 36, 41–50.

Wraight, S.P., Carruthers, R.I., Bradley, C.A., Jaronski, S.T., Lacey, L.A., Wood, P., Galaini-Wraight, S., 1998. Pathogenicity of entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. Journal of Invertebrate Pathology 71, 217–226.